

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Inventor: Wayne V. Vedeckis *et al.*
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Serial Number: 10/ _____
Title: Human Glucocorticoid Receptor 1A Promoter and Splice Variants
Attorney File: Vedeckis 97M20-D

Mail Stop Patent Application
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

PRELIMINARY AMENDMENT

Dear Sir:

Please amend the application as follows:

In the Specification:

At page 1, line 11 please insert:

-- This is a divisional of copending application serial number 09/552,619, filed April 18, 2000, now allowed with issue fee paid.--

The abstract has been amended as shown in the attached pages.

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In the Claims:

Please delete claims 5-6 and 8-13.

Remarks

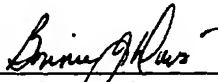
The specification has been amended to claim divisional priority from a prior non-provisional application.

The abstract has been amended to comply with 37 C.F.R. § 1.72(b). Changes in the text of the abstract are supported, for example, by the specification, page 6, lines 19-20; page 8, lines 21-23; and in the abstract as originally filed.

Claims 5-6, 8-13 have been canceled. Claims 1-4, 7, and 14-22 remain in the application.

Examination of Claims 1-4, 7 and 14-22 are respectfully requested.

Respectfully submitted,



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Revised Claim Listing

1. (Original.) A hGR 1Ap/e gene of the human glucocorticoid receptor promoter 1A and exon 1A comprising at least 2056 bases of SEQ ID NO: 1.
2. (Original.) A hGR 1Ap/e gene as in Claim 1, wherein the promoter region comprises the region from -1075 to -1 of SEQ ID NO: 1 as numbered in Figure 1.
3. (Original.) A hGR 1Ap/e gene as in Claim 1, wherein the exon region comprises the region from +1 to +981 of SEQ ID NO: 1 as numbered in Figure 1.
4. (Original.) A human glucocorticoid receptor exon 1A region as in Claim 3, wherein transcription of the exon region results in a mRNA transcript.
5. (Canceled.)
6. (Canceled.)
7. (Original.) A mRNA transcript of human glucocorticoid receptor exon 1A region as in claim 4, wherein the transcript results from transcription of the region +1 to +981 of SEQ ID NO: 1 as numbered in Figure 1.

8. (Canceled.)

9. (Canceled.)

10. (Canceled.)

11. (Canceled.)

12. (Canceled.)

13. (Canceled.)

14. (Original.) A method to increase the expression of mRNA transcript as in Claim 7 to treat a patient with T-cell acute lymphoblastic leukemia cells, comprising administering to the patient an enhancing amount of an exogenous demethylating agent to reactivate the human glucocorticoid promoter and exon 1A activity.

15. (Original.) The method of claim 14, wherein the demethylating agent is 5-azacytidine.

16. (Original.) A hGR 1Ap/e promoter-heterologous gene construct comprising all or a portion of SEQ ID NO:1 and a heterologous gene, wherein expression of the heterologous gene of the construct is under transcriptional control of the hGR 1Ap/e promoter.

17. (Original.) The method of claim 16, wherein the heterologous gene codes for a toxin.

18. (Original.) A method to kill targeted cells by administering an exogenous dose of glucocorticoid, comprising transforming targeted cells by introducing into said cells the gene construct of claim 17.

19. (Original.) A method to convert glucocorticoid-resistant lymphoblasts to glucocorticoid-sensitive lymphoblasts, comprising introducing all or a functional portion of SEQ ID NO: 1 into the hormone-resistant lymphoblasts.

20. (Original.) An antisense transgene comprising all or a functional portion of the promoter region of SEQ ID NO: 1 linked to a fragment of the exon region of SEQ ID NO:1 in the antisense orientation.

21. (Original.) A method to inhibit hGR1A GR mRNA from being up-regulated in cells, comprising introducing into said cells the antisense transgene of Claim 20.

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22. (Original.) A method to prevent neuronal apoptosis caused by excessive glucocorticoid secretion, comprising introducing into said neuronal cells the antisense transgene of Claim 20.

REVISED ABSTRACT

A new sequence, hGR 1A_{p/e}, has been isolated from human DNA upstream from the previously known 2.7 kbp human GR promoter region. This new sequence was found to contain a new promoter (the 1A GR promoter) and a new untranslated exon sequence (GR exon 1A) for the human glucocorticoid receptor protein (hGR). ~~The hGR 1A_{p/e} sequence is approximately 25 kilobase pairs upstream of the hGR coding sequence.~~ Alternative splicing produces three different hGR 1A-containing transcripts, 1A1, 1A2, and 1A3. ~~[GR transcripts containing exons 1A1, 1A2, 1B, and 1C are expressed at various levels in many cancer cells and in the human brain.]~~ Exon 1A3-containing GR transcripts appear to be restricted to blood cell cancers and to the human brain. Glucocorticoid hormone treatment caused an up-regulation of exon 1A3-containing GR transcripts in T-lymphoblast cells, and a down-regulation of exon 1A3-containing transcripts in B-lymphoblast cells. ~~This reaction correlates with the known response of the two cells to glucocorticoid hormone treatment; i.e., B-lymphoblast cells are known to be resistant to glucocorticoid hormone treatment and T-lymphoblast cells are known to be sensitive. Thus the presence of exon 1A3-containing transcripts can be used to detect cancerous blood cells that would be sensitive to glucocorticoid hormone treatment.~~ Additionally, an interferon regulatory factor element (IRF-E) that binds IRF-2 was found in the exon 1A sequence. ~~This regulatory factor appears to contribute significantly to basal transcription rate of 1A GR transcripts. The intraexonic location of this sequence was surprising. A glucocorticoid response element (GRE) was also found intraexonically in the exon 1A sequence. The presence of these two regulatory factors indicates that both interferon and glucocorticoid hormone could be used to increase the level of exon 1A3-containing transcripts in the~~

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~~cells. There are ~1075 base pairs of hGR 1A promoter sequence, based upon the absence of these sequences in mRNA. There are ~981 bp of exon 1A sequence. The portions of the hGR 1A/c sequence that function as a eukaryotic promoter and intraexonic regions that increase promoter activity were identified based on reporter gene assays. The~~ Thus ~~detection of exon 1A3-containing transcripts can be used for the diagnosis of patients with blood cell cancers, including T-cell acute lymphoblastic leukemia (ALL) and other glucocorticoid-responsive cancers, and to identify patients that would benefit from glucocorticoid hormone treatment.~~